STUDIES ON CESTODE PARASITES OF FISHES. II. THE NERVOUS SYSTEM OF TYLOCEPHALUM DIERAMA SHIP-LEY AND HORNELL.¹

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Introduction.

The present work is the outcome of some fundamental problems which arose when I had to describe a Monozootic Cestode whose features appeared to be different from those of Gyrocotylidae. Fuhrmann's account of the Cestoda in Kükenthal's Handbuch der Zoologie (1930) does not mention the nervous system in the definition of Cestodaria, The definition takes into account only the disposition of the generative organs and the character of the larva. It is a well-known fact that in the case of many fish parasites the proglottides are capable of living freely in the alimentary tract after having become detached from the proglottid chain. The problem thus reduces itself to the question: How can one distinguish a free living proglottid from a monozootic I have seen in several cases immature free living proglottides and so, if the larva is not available, the differentiation of a free living proglottid from a monozootic Cestode becomes virtually impossible. It appeared to me that the only definite evidence that one could rely on for differentiation is the nervous system. Theoretically therefore a monozootic Cestode should have a closed nervous system consisting of a brain and a system of nerve cords while in the free living proglottid the brain should be absent. It was because I found a closed nervous system in Biporophyllaeus (Subramaniam 1939) that I described it as

¹ An abstract of this paper was read before the 28th Session of the Indian Science Congress held at Benares in January 1941.

belonging to a new order of Cestodaria. And it was on the same ground that I suggested that the Caryophyllaeidae should be included among the Cestodaria.

I have to state, however, that descriptive accounts of the nervous system of representative members of the various families of Cestode parasites of fishes are not available in the few papers published on the subject. Very little is known about the arrangement of the nerves in the proglottides and nothing about the changes in the nervous system following the separation of a proglottid from the chain. It has also to be mentioned here that very few of the older workers used any of the typical neurological technique so that even to-day the study of the nervous system of cestodes remains the step-child of Helminthologists.

I thought that investigations of the nervous system of Cestodes of fishes belonging to the various families would offer a solution to the difficulties mentioned above. The choice of material for an investigation of this sort is not, however, at the command of the investigator. Nothing is known about the extent, the rate and the time of incidence of the various fish parasites. It will be obvious, therefore, that one has to take his chance with the material available. Often it is likely that the investigator may not obtain another lot of material during the year. A systematic neurological investigation of representative members of the various families is therefore not possible. The only possible alternative is an investigation of such specimens belonging to different families as may be available, and arrive at some definite conclusions after a survey of the whole field when a sufficient number of representatives of the different families have been investigated. The following is a preliminary attempt in the direction outlined above.

MATERIAL AND METHODS.

In the present paper is recorded the investigations carried out on specimens of Tylocephalum dierama Shipley and Hornell, collected in July 1939 from Rhynchobatus djeddensis. About 250 specimens were available, out of which 50 were fixed in ammonia alcohol and chloral hydrate alcohol, 30 in Golgi's osmo-bichromate mixture, and the rest in 5 per cent formalin. Part of the material fixed in chloral hydrate alcohol and ammonia alcohol was treated with pyridine before transfer to silver nitrate. Golgi's rapid method and Cajal's methods were thorough failures. Material fixed in 5 per cent formalin was then treated according to the Bielchowsky method. For about 6 months the attempts to impregnate the fibrils and nerve cords were without success. It was then discovered that treatment with pyridine was essential. Finally, by manipulating the time in pyridine, silver bath and ammoniacal silver bath, some very good impregnations were obtained. was found that in light impregnations the nerve cords were brownish while the nerve fibres proceeding in various directions from the cord The vitelline glands being argentophile, obscured, in deeper impregnations, the finer details of the origin and direction of the fibrils leaving the nerve cords. Naturally, therefore, all the descriptions are based on examination of preparations impregnated in varying grades of intensity. Almost all workers on the nervous system

of tapeworms describe ganglia and commissures especially at the posterior end of the mature segments. No ganglionic thickenings were observed in the nerve cords of Tylocephalum, but the commissure at the posterior end of the segments was prominent. Since the ganglionic cells were indistinct in these preparations, a re-investigation with the Bielchowsky technique was found essential. Material silvered without previous treatment with pyridine was, in the majority of cases, useless. But, in a few, the ganglionic cells were impregnated. In such preparations the peripheral fibrils were unstained. There was also the question of the nature of arrangement of the muscles in the segments. Southwell (1925) in his Monograph on the Tetraphyllidea figures a row. of longitudinal muscles in the parenchyma. The first few series of Bielchowsky preparations suggested that what Southwell took for muscle bundles were nerve cords. Therefore material preserved in 5 per cent formalin was fixed in Susa and Bouin Duboscq, sections were cut, and part of the Susa material was stained with Mallory's triple An examination of the Mallory-stained sections revealed a subcuticular muscle system composed of outer circular and inner longitudinal fibres. The bundles of fibres which stained positively with Bielchowsky were light violet while the muscle fibres were light red. In such Susa-Mallory preparations, the fibrils proceeding to the various tissues, the circular commissures and the posterior plate commissure were light violet in colour.

The Bouin Duboscq and Susa material, when stained with iron haematoxylin, showed the subcuticular muscles blue, while the nerves were unstained and structureless in appearance. Counterstaining with eosin or orange G did not improve the appearance. When the Bielchowsky material was mordanted for 2-3 days in iron alum and stained in haematoxylin for 4-5 days the ganglionic cells could be seen in the nerve cords. In such slides the ganglionic cells themselves are deep black while the fibres to the various organs are brownish yellow. In some cases such preparations are found more suitable for photomicrography than the Bielchowsky preparations. The muscle fibres in these preparations are blue black and in those regions of the worms where the outer layers are slightly macerated, the longitudinal and circular fibres together give a lattice-work-like appearance (Pl. X, fig. 1). Thus in Tylocephalum dierama there is no medullary longitudinal or circular muscle layer.

The histological details were confirmed by Vom Rath preparations stained with iron haematoxylin by the long process. In spite of the large quantity of material available, the histology of the brain could not be investigated satisfactorily, probably due to the long treatment of the parasites with tap water. The worms occurred firmly attached to the folds of the spiral valve and they released their hold only after having been in tap water for 30-45 minutes. Attempts to remove them by force resulted in the proglottid chains snapping near the neck region. As repeated examinations for the past one year of the spiral valve of *Rhynchobatus* and other allied rays have failed to reveal any *Tylocephalum dierama*, a more detailed histological study of the brain has to wait till further material is available.

THE BRAIN.

The brain lies in the middle of the myzorhynchus (Pl. X, figs. 2, 3). In well expanded specimens the myzorhynchus is egg shaped and the brain lying in it has the shape of an arc. This is but a plane view. If reconstructed from serial longitudinal and transverse sections it is seen to have the shape of a conical cap. The posterior half of the brain presents a vacuolated appearance in Bielchowsky and Vom Rath longitudinal sections. In Vom Rath slides each of these vacuoles presents the appearance of a nucleus (Pl. X, fig. 4, nc.). Some of these show distorted nucleoli while in others a tangled mass of chromophile threads may be seen. The cytoplasm covering these nuclei is thin and not very clear and small bundles of fibres could be seen running antero-posteriorly between the nuclei. Plate X, figure 3 shows the innumerable bundles proceeding from the brain fan-wise to the anterior edge of the myzorhynchus.

A transverse section of the brain shows that it is a plate-like struc-In Bielchowsky preparations (Pl. X, fig. 5) radiating fibres could be seen proceeding in a radial direction all round. Careful examination of the section under oil immersion shows innumerable very minute These occur in rows. A Vom Rath transverse section gives a slightly better idea of the histology of the brain (Pl. X, figs. 6, 7, nc.). The nuclei appear to be of two types: (1) large ones as seen in longitudinal sections and (2) small ones arranged in a linear manner. rows of nuclei separated from one another by small bundles of fibres are arranged in the shape of concentric arcs. Plate X, figure 7 gives a very good idea of the arrangement. As in the case of the larger ganglion cells these minute nuclei have only a very thin cytoplasmic coat. nucleolus is present in many of these nuclei. Nerves to suckers arise separately from the brain. From transverse and longitudinal sections it appears that the sucker is constituted by one layer of cells. outer half of the cells in Bielchowsky iron haematoxylin preparations appears darkly stained. The basal half is unstained and nuclei occur. about the middle of the clear half.

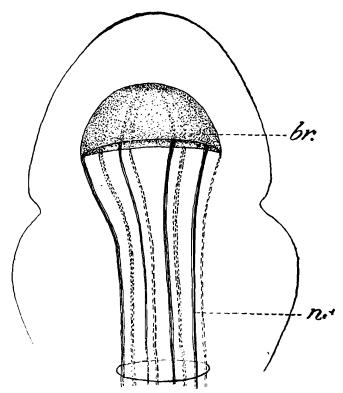
In silvered preparations the nerve proceeding from the brain is observed to touch the anterior inner border of the sucker. The base of the sucker has an investment of fibrils and from this meshwork fine fibres could be seen proceeding between the cells.

To sum up: It is observed that the brain is not constituted by a system of ganglia but is plate-like, the slight differences in shape depending on the degree of contraction of the head and myzorhynchus. The nerve cords take their origin from the posterior margin of the brain. Bundles of fibres proceed anteriorly and get distributed at the anterior edge of the myzorhynchus. There are large and small ganglion cells in the brain.

NERVES IN THE DILATED PORTION OF THE HEAD AND THE NECK.

The number of nerves starting from the brain could not be definitely mentioned as the bundles lie close together and merge into one another near the brain. In transverse sections (Pl. X, fig. 8) 8-14 bundles of nerves could be seen in the region of the sucker, each bundle being composed of 2 or more nerves. In some bundles it is possible to count the number of nerves constituting the bundle but in others they are so closely packed as to present the appearance of a single nerve.

In text-figure 1 is given a diagrammatic representation of the brain and the arrangement of the nerves in the dilated portion of the head.



Text-fig. 1.—A diagrammatic representation of the brain and the nerves in the neck region of *Tylocephalum dierama* Shipley & Hornell. br., brain; n., nerve cords.

The neck region is very short and transverse sections show a variable number of nerves ranging from 32 to 42.

NERVES IN THE EARLY SEGMENTS.

The nerve cords in the early segments are oval in transverse sections and have a diameter varying from 8.3 μ to 18.3 μ along their longest axis which has a radial direction. In frontal and sagittal sections passing near the surface, where in a single section several nerve cords may be observed lying side by side, fine fibres are seen running from one cord to another (Pl. X, fig. 9) at irregular intervals presenting a ladder-like appearance. At the hind end of the early segments the commissure is virtually absent. But in segments about 4 mm, from the posterior border of the head, the commissure could be observed as a few fibres.

In Bielchowsky iron haematoxylin material the neurones are deeply stained. A differentiation of such cells into nucleus and cytoplasm is however impossible. Either the whole neurone is yellow or it is stained blue black. The ganglionic cells appear to be of three types. Most of them are bipolar but what look like unipolar and multipolar ones are also met with (Pl. X, fig. 10, mg.).

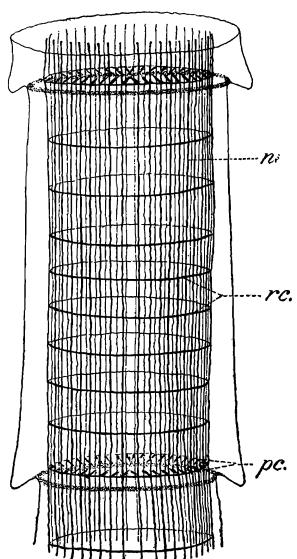
Compared to the maturing proglottides (Pl. XI, fig. 1), the neurones in the early segments seem to be more closely packed together. Though in general there is no marked concentration of ganglionic cells in any particular region of the nerve cerd in a segment, occasionally cells are concentrated in particular regions, as for instance, near the posterior margin of the segments. Here unipolar and multipolar cells are found to be present. These cell aggregations do not, however, touch the posterior wall of the segment. Rarely aggregations are seen in the nerve cord about the middle of the segment.

In some regions of the nerve cords unipolar ganglion cells dominate. They are thinner than the bipolar ones, but longer. The nerve cord has a yellowish appearance in Bielchowsky iron haematoxylin preparations due to the large number of brownish nerve fibrils. Some of the ganglionic cells have a curved contour, one of the ends of the cell body touching the lateral margin of the nerve (Pl. XI, fig. 2, nc.). From this end the fibre could be traced to the tissue or organ innervated. from the portion of cell body parallel to the nerve cord gets mingled with the other fibres. The fibres of some of the bipolar ganglion cells which are disposed lengthwise in the nerve cord could be seen leaving the latter at some distance. Binding cells are absent and therefore the nerve cords are loose in structure. Scattered ganglion cells are often found outside the nerve cord running parallel to the nerves, between them and the cuticle. The longest diameter of the nerves in early segments varies from 8 μ to 18 μ , the body of the bipolar neurones measure from 7 μ to 15 μ in length and 1.66 μ to 3.3 μ in width and the multipolar neurones vary from 5-17 μ in length and 2-4 μ in width. In the early segments the main nerve cords do not give rise to nerve branches. Innervation is by nerve fibrils which leave the cords at irregular intervals. In the very early segments succeeding the neck region few of the organs are differentiated and one does not see the complicated tangle of nerve fibrils observed in maturing segments.

It is surprising to find on examination of several series of sections that the number of nerves is always even, if not constant and varies from 32 to 42. The usual number, however, is 38. It will be observed that while dealing with the nerves of the neck and the very early segments the same variation in number was mentioned. What strikes one in a transverse section is the constant occurrence of commissures connecting the nerve cords (Pl. XI, figs. 3, 4). This has actually the appearance of a circle of tangled fibres, the nerves forming knots in this circle. The most prominent commissure is at the hind end of each segment near the septum (Pl. XI, fig. 5).

The ring commissure appears to be formed by fibres from one nerve cord passing on to those on either side of it. The number and complexity of these ring commissures increase with the maturity of the segments, and in a mature segment itself the circular commissures are more abundant in the posterior half. The plate commissure at the posterior end of the segment is formed by a meshwork of fibrils. The meshwork is loose in the middle segments and compact in the later and mature ones. The lateral margins are strongly salient or imbricated and overlap the anterior end of the succeeding segment. Therefore the nerve cords occur at different depths from the cuticular margin

in different regions of the same proglottid. At the anterior end of each proglottid there appear to be very few layers of subcuticular cells between the nerve cord and the cuticle, and passing backward they have a deeper disposition especially near the posterior margin. Near the imbricated edge one bundle of fibres leaves each of the nerve cords to the cuticle at the level of the posterior plate commissure (Pl. XI, fig. 6). Nerve cells are not found in this branch which appears to correspond to the marginal nerve described by Tower (1900). This nerve does not proceed even half way up the segment but its fibres separate fan-wise and come into contact with the sense cells lying midway between the nerve cords and the cuticle in the subcuticular layer. Due to the salient posterior margin the septa between the segments are not exactly transverse but are semi-circular. In sections therefore we see only portions of the plate commissure (Pl. XI, fig. 5, pc.).



Text-fig. 2.—A diagrammatic representation of the nerves and commissures in a mature segment of Tylocephalum dierama Shipley & Hornell.

n., nerve cords; pc., plate commissure; rc., ring commissure.

NERVES IN THE MATURE SEGMENTS.

As the generative organs mature and the segments increase in size and thickness, the nerve cords, which had their longest axes disposed

in a radial direction in transverse sections, become thinner, lose their oval shape, and assume an irregular appearance. Even the thickness of a nerve cord varies from region to region in the same segment.

Binding cells are absent and most of the ganglion cells are bipolar. Multipolar cells are present but unipolar ones are scarce. The measurements of these cells fairly agree in their range with those in the early segments. Here also very large ganglion cells, 17 μ by 3 μ , are occasionally met with. In Bielchowsky iron haematoxylin preparations the ganglionic cells stain blue black, and in many longitudinal sections single ganglionic cells lying outside, but parallel to the nerve cords, may be observed.

From Vom Rath preparations it appears that the nuclei of multipolar ganglion cells are almost round and have a diameter of 2.0μ . The round nuclei of bipolar cells measure 0.83μ to 1.66μ and the oblong nuclei of the large bipolar cells 6.6μ by 4.2μ .

In Bielchowsky preparations the nuclei of the neurones are dark brown while the cytoplasm is yellow. The nucleus in bipolar cells is slightly oval, and the biggest measures 2.0μ . The biggest unipolar ganglion cells is 10μ long and 1.7μ wide. The average size of nuclei in multipolar cells is 2.5μ .

Text-figure 2 is a diagrammatic representation of the arrangement of the nerves and commissures in a mature segment.

INNERVATION OF THE VARIOUS ORGANS.

It will be seen from the foregoing account that the nerves form a cylinder enclosing the various organs in the medulla. Separate nerves to groups of organs or tissues were not observed and each organ is innervated by nerve fibrils having their origin in different nerve cords lying in the same sector containing the organ. In order to give a clear idea of the mode of innervation I shall describe first the distribution of nerve fibrils to the ovary.

(a) The Ovary.—The ovary is a globular organ, $\frac{1}{6}$ to $\frac{1}{8}$ as long as the mature segment, occupying the hinder end of the segment. It is really composed of two lobes which lie touching each other. The bilobed appearance, however, is very marked in the centre of the organ where the two lobes are caved in to lodge the shell gland. The organ is composed of radiating acini which differ in size among themselves. Owing probably to the removal of fat in the occytes, during fixation and dehydration, these acini show only irregular scattered nuclei inside, which, with bits of cytoplasm sticking to them, represent the occytes.

Plate XI, figure 7 is a longitudinal section showing the fibres leaving one of the nerve cords. A number of separate fibres leave the nerve cord near the junction between two acini and running along the junction either terminate in the acinus itself or proceed to the tissues and organs in the interior. As the nerve fibrils seem to take a wavy course and as there is a mingling of the fibres having their origin in different nerves or different portions of the same nerve, very few of the fibres proceeding to the inner regions could be traced along their entire course. Though no ganglionic cells occur in the posterior plate commissure,

yet in longitudinal sections nerve fibrils could be seen leaving the commissure and running between two acini.

Plate XI, figure 5 is a transverse section from a Bielchowsky preparation. It shows the fibres leaving the various nerve cords and their course in the ovary. In the dorso-ventral space between the two lobes of the ovary large numbers of fibres may be seen proceeding towards the middle. These fibres start from nerves lying near that region. The fibres from the nerves not directly above the space between the two halves of the ovary take a curved course and are joined by fibrils from the nerves lying just above it.

(b) The Testicular Vesicles.—The above description makes it easy to understand the mode of innervation of the other organs, as it is identical in all organs.

The segments of the middle region of the worms are packed with testicular vesicles; the vitelline glands and ovary have not, however, begun to develop. Most of the testicular vesicles show fully developed sperms. Plate XI, figure 8 from a Bielchowsky preparation shows how irregular is the course of the nerve fibres from the nerve cords. The testicular vesicles are matted over with nerves fibrils, which, are shown by transverse sections, to arise from all the nerve cords.

Complex networks of fibrils occur also in regions where vitelline glands and ovary develop later. In segments where the vitelline glands and ovary are well developed the testicular vesicles are pushed into the middle owing to the development of the vitelline glands at the two sides. Some of these testicular vesicles extrude their contents and then degenerate, and in a mature segment one of the testicular vesicles lying near the cirrus pouch is considerably bigger than the others. Transverse sections show that these vesicles lying in the medulla are innervated by fibrils originating from the nerve cords lying in the same are as the vesicle. In many cases it was observed that the bundle proceeding to the testicular vesicles was composed of many fibres, though coming from different cords.

(c) The Vitelline Glands.—After the completion of the spermatogenesis in most of the testicular vesicles, the vitelline glands begin to develop. In the mature segments they are well developed and massive and extend in some cases along the sides of the ovary to the few acini which occur in almost all segments behind the ovary.

Plate XI, figure 9 shows the innervation of the vitelline glands. It is a longitudinal section and shows the fibrils leaving a single nerve cord, while Plate XI, figure 4 shows how fibrils from a number of nerve cords take part in the innervation of an acinus.

SENSE ORGANS.

In deep impregnations many of the fibrils leaving the nerve ring or nerve cords seem to end near nuclei lying midway between the nerve cords and the cuticle. These nuclei appear to be those of the sense cell as shown previously by Blochmann (1895). Under an oil immersion, fibrils from these nuclei could be seen taking a wavy course and ending in knob-like swellings on the inner edge of the cuticle. Irregular precipitation of silver occurs on the cuticle in deep impregnations and in

most cases these end knobs are indistinct. In light impregnations they could be seen as triangular or egg-shaped structures yellowish brown in colour. Due to the wavy course of the fibrils from the sense cells, the whole fibril is not in the same plane and hence more than one photograph is required to show the fibril connections of the sense cell to the nerve ring on the one hand and the cuticle on the other. Plate XI, figure 10 shows this relation. The cytological structure of the sense cells is not very clear. Fibres going directly to the cuticle without any contact with sense cells have also been observed.

Discussion.

In order to compare the nervous system of Tylocephalum dierama described above, a knowledge of the systematic position of the worm Tylocephalum possesses a typical Cyclophyllidean head, is essential. but the genitalia resemble in arrangement those of the Tetraphyllidea. This mixture of the Tetraphyllidean and Cyclophyllidean characters has led to its being included in either of the above orders. Meggitt (1924) and Fuhrmann (1930) included Tylocephalum in the order Tetraphyllidea. and separated the Cyclophyllidea from all the other orders on account of the compact and unpaired nature of the vitelline glands. Southwell (1925) on the other hand included it among the Cyclophyllidea arguing that if the globular portion of the head in Tylocephalum has arisen as a result of the fusion of the bothridia one might also assume that a similar process has occurred in the whole of the Cyclophyllidea. various species of the genus itself show variation. In Tylocephalum dierama the vitelline glands occur as two massive organs whose acini extend on either side up to the anterior end of the ovary, and in many cases even to the posterior end of the segment. But in T uarnak the vitelline glands are single and very small and placed behind the ovary. From the above it would appear that Tylocephalum may possibly be a connecting link between Tetraphvllidea and Cyclophyllidea.

Cohn (1900)¹ as a result of his researches concludes that the primitive form of the nervous system which must have governed the common ancestors of Cestodes should have been an irregular network traversing unbrokenly the entire body of the Cestodes. He states that in all the Cestodes studied by him the two main longitudinal stems have already been differentiated and therefore considers the nervous system of Liquia as the simplest of the extant types. Ligula and Schistocephalus have two main longitudinal stems and a much larger number of longitudinal nerves than any other Cestode. The main reason for the belief that they occupy the lowest position is that all the longitudinal nerves—the two main ones excepted—are exactly similar and without any secondary differentiation. Cohn argues therefore that the meagre adaptation of these animals to intestinal parasitism is the cause for the absence of any differentiated development in these animals, and this seems to be substantiated by the insignificant development of the sucker grooves and the resulting simplicity of the scolex innervation.

¹ My thanks are due to Mr. N. T. Mathew, M.Sc., for help in translation of the paper.

It will be seen that the nervous system of Tylocephalum is more primitive than that of Ligula and Schistocephalus. The brain is a capshaped plate from which arise 32-42 nerves. As in Ligula and Schistocephalus there is an irregular meshwork of nerve fibrils at the very anterior end of the proglottid chain, though further behind the meshes have differentiated themselves into ring commissures. But Cohn's explanation that the primitive nature of the nervous system of Liquia and Schistocephalus is due to their meagre adaptation to intestinal parasitism does not hold good in the case of Tylocephalum. Development of suckers on the head is a distinct adaptation to parasitism, but why Tylocephalum has a simpler nervous system than Liquia is at present inexplicable. The question whether the nervous system of Tylocephalum is the simplest in the polyzootic Cestodes cannot also be answered until many more members of the different families of Cestodes are investigated. As simplicity of arrangement of the nervous system does not indicate poor adaptation in Tylocephalum it is likely that further exhaustive studies may alter entirely the present conceptions of the phylogenetic relationships among the various orders of Cestodes.

SUMMARY AND CONCLUSIONS.

The nervous system of Tylocephalum dierama is very simple in plan. The brain is not constituted by a system of ganglia but is plate-like and the slight differences in shape observed in different examples are due to the various degrees of contraction of the head and myzorhynchus. Bundles of fibres proceed from the brain and get distributed at the There are large and small ganglion anterior edge of the myzorhynchus. Thirty-two to forty-two nerves, all of the same cells in the brain. thickness, run through the length of the proglottid chain. A meshwork of nerve fibrils connecting the nerve cords is present at the extreme anterior end of the proglottid chain but further behind the meshes have differentiated themselves into ring commissures. The commissure at the posterior end of each proglottid is plate-like. The nervous system of Liquia and Schistocephalus is supposed to be the most primitive as the insignificant development of the sucker grooves in these animals indicates a poor adaptation to parasitism. These animals have two main lateral stems and a far larger number of longitudinal nerves than any other Cestode. In Tylocephalum dierama all the nerves are of the same size, and therefore it is assumed that its nervous system is more primitive than that of the two above mentioned animals. simplicity of arrangement of the nervous system does not indicate poor adaptation in Tylocephalum—as evidenced by the presence of suckers -it is likely that further exhaustive studies may alter entirely the present conceptions regarding the phylogenetic relationships among the various orders of Cestoda.

ACKNOWLEDGMENT.

I wish to express my grateful thanks to Professor R. Gopala Aiyar for his criticisms and encouragement.

EXPLANATIONS OF LETTERING IN PLATES.

Br., Brain; cm., Circular muscles; lm., Longitudinal muscles; mg., Multipolar ganglion cells; mn., Marginal nerve; n., Nerve cord; nc., Nerve cells; nf., Nerve fibrils; oa., An Acinus of the ovary; pc., Plate commissure; rc., Ring commissure; sc., Sense cells; tv., Testicular vesicles.

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